A NOTE CONCERNING THE DISTRIBUTION OF POLYSACCHARIDES IN THE EARLY DEVELOPMENT OF THE HYDROMEDUSAN PHIALIDIUM GREGARIUM [1, 2]

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Since many organisms are actually supported by polysaccharides, that is, they form the framework for the architecture of the plant or animal, it was thought that it would be most interesting to follow the distribution of polysaccharides during the course of development, from egg to polyp, in the hydromedusan *Phialidium*. Fortunately, there is a simple and dependable histochemical technique to demonstrate these substances, namely, the periodic acid-Schiff method and, as will be shown, the non-starch polysaccharides are present in large amounts throughout the development and no special region of secretion of the external chitinous perisarc can be seen. On the other hand, there is a most striking and curious pattern associated with the attachment process of the planula larva.

Methods

The jellyfish were collected in the evening at a night light off the dock of the Laboratory of the University of Washington, at Friday Harbor, in Puget Sound. They were immediately placed in fresh sea water in finger bowls (100 mm. diameter) on the water table and kept at approximately 13° C. Six to a dozen jellyfish were placed in each bowl and these were removed the next day so that the eggs which had been shed could develop. For the attachment stages, two per cent agar in sea water was prepared to cover the bottom of clean finger bowls. Early planulae were transferred to these dishes, and when they attached to the agar, they could be easily removed without being damaged and transferred to the fixative.

The embryos were fixed in Bouin's fluid at 2° C., dehydrated in a series of graded alcohols, and embedded in paraffin. Polysaccharides were demonstrated by the periodic acid-Schiff technique (Gomori, 1952). The best results were obtained when a three-hour period of salivary digestion, which removes all the glycogen, preceded the staining. Control slides were run without the oxidation which showed an evenly distributed, very faint pink tinge.

[1] These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and J. T. Bonner, NR 164–274.

[2] It is possible that some of these observations were made on individuals of the closely related species, *P. hemisphericum*, for it is likely that the two species are both present in Puget Sound. Specific characters include the numbers of tentacles and marginal vesicles, and since these structures vary within rather wide limits, certain identification rests upon the examination of large numbers of individuals and the rearing of the hydroid stages.

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Results

As can be seen from Figure 1, in the egg there is a fairly even distribution of non-starch polysaccharide located primarily in large granules and this same pattern persists throughout cleavage as well as in the blastula stage. Even in the beginning of gastrulation, as the future endoderm cells wander into the blastocoel at one pole, there is no major difference among the cells in their staining intensity.

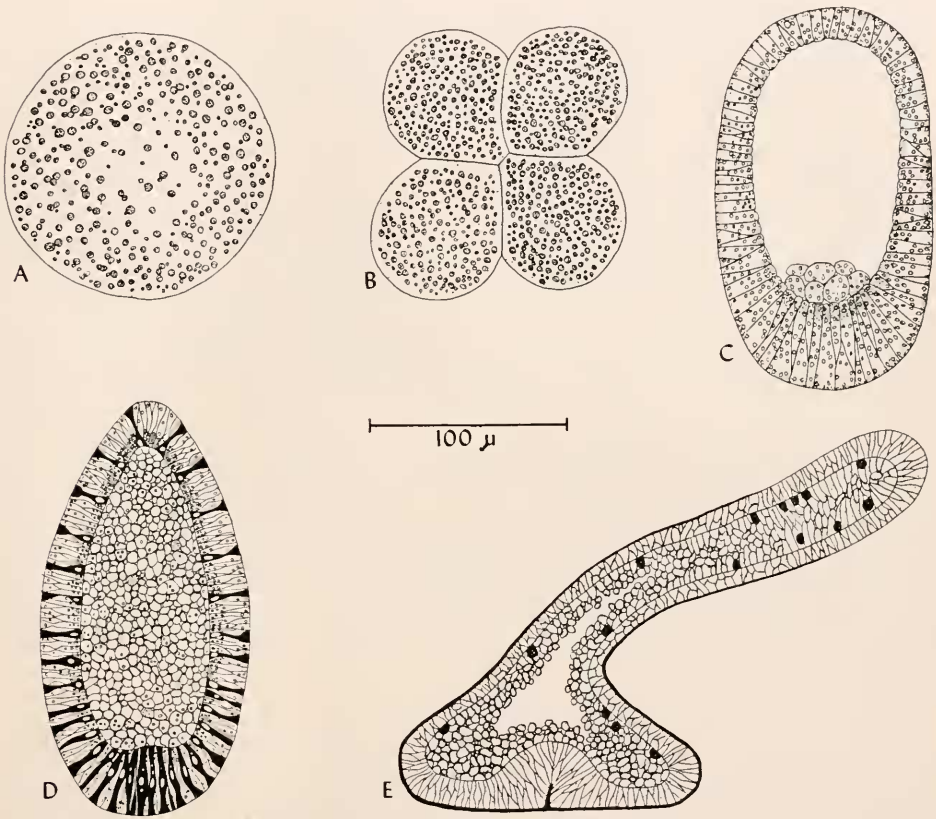

Figure 1. Drawings showing the distribution of non-starch polysaccharide in the development of *Phialidium*. The intensity of the black reflects the concentration of the polysaccharide. A, egg; B, 4-cell stage; C, 23 hours after fertilization showing the beginning of gastrulation by polar ingression (note that in this drawing and the next, the anterior end which attaches to the substratum is pointing downward); D, a late planula larva 60 hours after fertilization; E, a young attached larva just prior to the appearance of annulations in the chitinous perisarc.

It is only well after the gastrulation is complete and the motile planula shows contractile worm-like movements and ciliary activity, that there is a beautiful pattern of intensely stained goblet cells distributed within the entire ectodermal layer.

These cells are clearly more concentrated in the blunt anterior pole, in fact, in some larvae they appear so dense in that region that they almost lie side by side in a solid mass. The tapering, posterior end (which will later become the

hydranth end of the developing polyp) has few or none of these cells and there is a gradient of increasing goblet cell concentration as one passes anteriorly. In a surface view it is striking to see that these trumpet-shaped cells are spaced in an orderly fashion, each occupying the position farthest removed from all its neighbors. At this stage there are no especially intense staining regions in the endoderm.

Upon fixation the planula attaches its anterior end to the substratum and proceeds rapidly to flatten like a pancake. Almost immediately, hard chitinous material is secreted about this flattened mass and then some hours later a small nipple appears at the location that was the posterior end. This nipple, which is of a fixed diameter, continues to elongate (probably largely by mass cell migration or morphogenetic movement), showing annulations in the perisarc as it proceeds, and this is the stem or coenosarc of the future primary hydranth that eventually appears at the apical end.

Once fixation was accomplished and the first chitinous material surrounded the flattened embryo, there was no evidence of the intensely stained trumpet-shaped cells at all. They had apparently given off all their material in the fixation process, and we might assume from this that these gland cells have during the course of evolution been perfected in the planula solely for the function of fixation. The fact that planulae possess gland cells is well known and described in the early literature (see, for example, Wulfert, 1902) but their orderly distribution over the surface, their activity in relation to fixation and the production of polysaccharides was never specifically examined.

It is particularly surprising that the rounded tip of the young coenosarc which rises upward, does not have a special zone of secretion, for it is there that the chitinous wall is primarily deposited. The cells of this rising nipple appear evenly stained and, therefore, are high in polysaccharide content but they have no special gland cells nor any clearly defined region of deposition. In the endoderm, lying near the mesoglea, there are round masses of more intensely stained material, but because of their internal location, it is hard to understand what function they might have.

Summary

The distribution of non-starch polysaccharides was studied from egg to attached polyp in the hydromedusan *Phialidium gregarium* and it was found that the polysaccharides were fairly evenly distributed throughout the development except in the late planula. There a number of gland cells richly supplied with polysaccharide appear in the ectoderm and show a gradient—sparse in the posterior end and dense in the anterior attachment region. These cells are apparently concerned with fixation, for after the larva has attached and secretes a hard chitinous covering, these ectodermal concentrations of polysaccharide are no longer visible.

LITERATURE CITED

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